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(54) Title: USE OF AN ENZYME FOR PROMOTING PIGMENT UPTAKE FROM A FEED

(57) Abstract

Provided is the use of an enzyme selected from a carbohydrase and/or protease as a component of a non–viscous animal feed for promoting the absorption of pigments present in the feed. Non–viscous animal feeds are based on cereals such as sorghum, rice and maize, on oilseeds such as soya, canola and rapeseed, or on root crops such as tapioca and cassava. The pigments may be either present naturally in the feed or added to the feed and include such carotenoid pigments as astaxanthin, canthaxanthin, lutein, zeaxanthin and β–apo–8'–carotenal. Also provided is a non–viscous animal feed and a method for increasing the bio–availability of a pigment present in an animal feed by incorporating 1×10^{-6} – 1 % by weight of a carbohydrase and/or a protease into the feed.

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USE OF AN ENZYME FOR PROMOTING PIGMENT UPTAKE FROM A FEED

The present invention relates to the use of an enzyme for promoting pigment uptake from a feed by an animal, and in particular such a use which enhances the absorption of carotenoid pigments present in a feed by animals such as fish, crustaceans and healthy poultry.

Improvements in animal feeds to enable animals to digest their various nutritive and bio-active components more efficiently are constantly being sought. This is particularly so for relatively expensive bio-active ingredients specifically incorporated into the feed in order to have a desirable effect on the animal. One such group of additives are pigments, and in particular carotenoid pigments, which are routinely incorporated in feeds formulated for broiler chickens, layer hens, some fish species and crustaceans. These pigments are added in order to meet consumer demand as to the colour and appearance of food, and in particular to improve broiler skin pigmentation, egg yolk pigmentation, flesh and skin pigmentation of fish and crustacean flesh pigmentation.

The colour of carotenoid pigments ranges from yellow through to red. These pigments include astaxanthin, canthaxanthin, lutein, zeaxanthin and xanthophylls. These various pigments are derived from microbes, plants, animals or by synthetic means. For example, astaxanthin can be produced by synthetic means, from the yeast strain *Phaffia rhodozyma*, from the algae strain *Haematococcus pluvialis* and from crustacean by-products. Canthaxanthin can also be produced by synthetic

means, from crustacean by-products, bird feathers, and microbial sources such as *Cantharellus cinnabarinus* and *Rhodococcus maris*. Carotenoid pigments may also be produced by means of the genetic engineering of microorganisms such as described in EP-A-0 393 690 and WO 91/13078.

It has long been established by experimentation that different sources and types of carotenoid pigments are absorbed with different efficiencies by animals. In general though typical rates of absorption are relatively low. For instance, it is generally accepted that only 5-10% by weight of astaxanthin in a fish food is absorbed by salmonids. Even lower rates of absorption for astaxanthin present in feeds for chickens are normally found. A much higher absorption rate of 24-36% by weight for canthaxanthin has been reported. But still 42-54% by weight of dietary canthaxanthin is found to be excreted in the faeces and therefore wasted.

It is therefore a well established problem that absorption of pigments present in animal feeds is relatively poor. This is particularly disadvantageous bearing in mind the relatively high cost of pigments which are incorporated into animal feeds compared to the rest of the components of the diet. For example, in a broiler or fish feed, the pigment may constitute up to about 0.5% by weight of the feed, but be responsible for around 15% of the feed cost. Accordingly, it would be economically advantageous to provide means for ensuring improved absorption of pigments by an animal from its feed.

It is already known to include enzyme supplements in animal feeds. For example, GB-A-2 287 867 discloses the addition of a xylanase to an animal feed for assisting live-stock to digest protein and/or amino acids present in the feed. WO

96/05739 discloses a cereal-based feed comprising at least 25% by weight of wheat, a xylanase, a protease, and optionally a β-glucanase. The presence of these various enzymes improves the feed conversion ratio and/or increases the digestibility of the cereal content of the feed. Whilst both of these references provide detailed examples of certain animal feeds, none of these feeds contains a pigment nor are pigments mentioned in general in either of these references. These references relate primarily to cereal-based animal feeds which are categorised as being viscous being based upon wheat, rye, triticale, barley or oats. Cereals of this type contain a relatively high level of soluble non-starch polysaccharides which result in the content of the animals' gut being relatively viscous.

In contrast to the above mentioned viscous animal feeds, non-viscous animal feeds are also known being based upon cereals such as sorghum, rice, millet or maize, oil seeds such as soya, canola and rapeseed, or root crops such as tapioca or cassava. These do not contain a significant content of soluble non-starch polysaccharides and so do not cause the content of the animals' gut to be so viscous. As excessive gut viscosity is one of the main factors which prevents efficient digestion, it could not have been predicted that adding enzymes to non-viscous animal feeds would have any substantive effect on the absorption of bio-active materials such as pigments from them.

WO 91/04673 discloses a feed additive for treating poultry suffering from the disease malabsorption syndrome. The additive comprises a combination of a cellulase and a xylanase which may be obtained from *Trichoderma longibrachiatum*. It is noted that such a treatment of diseased birds results in

improved pigment uptake from a maize-based feed. There is no indication in this reference that such a feed additive would have a beneficial effect when included in the diet of healthy poultry.

It is an object of the present invention to provide the use of an enzyme for promoting the absorption of pigments present in a non-viscous animal feed.

Accordingly, the present invention provides the use of an enzyme selected from a carbohydrase and/or a protease as a component of a non-viscous animal feed for promoting the absorption of pigments present in the feed by fish, crustaceans or healthy poultry.

The term "healthy poultry" used herein refers to poultry which are free of at least the disease of malabsorption syndrome.

It has been found that the inclusion in a non-viscous animal feed of a carbohydrase and/or a protease enables the animal to absorb pigments present in the feed more efficiently. Thus the addition of such enzymes increases the proportion of pigments digested by the animal. As a result of this surprising finding, it is possible to modify previously used feeds by reducing their pigment content whilst simultaneously maintaining the same level of pigments absorbed by the animal. This means that the amount of costly pigments conventionally included in an animal feed can be reduced as compared to previously used feeds. This results in a significant reduction in the cost per unit weight of animal feed without decreasing its pigmenting ability. Alternatively, the same amounts of pigments as previously used can be maintained in accordance with the use provided by the present invention with

the benefit that enhanced levels of pigment are absorbed by the animal leading to products having higher market value due to e.g. enhanced skin tone in a broiler chicken which some consumers find attractive.

It is preferred that the non-viscous animal feed comprises at least 15% by weight, preferably 25-70% by weight, of one or more of:

(i) a cereal selected from sorghum, rice, millet, maize and a by-product of any thereof,

(ii) oilseeds selected from soya, canola, rapeseed, pea, bean, peanut, sunflower, safflower, lupin, flax and a by-product of any thereof, and

(iii) a root crop selected from tapioca, cassava and a by-product of either thereof.

Whilst it is possible that the desired pigments may naturally be included amongst the components making up the feed, such as a yellow pigment in maize which results in chickens fed maize having a pleasing yellow skin coloration, it is also possible to incorporate additional amounts of pigments into the feed. Such pigments are preferably carotenoid pigments such as astaxanthin, lycopene, β-carotene, citraxanthin, capsorubin, β-apo-8'-carotenoic acid ethyl ester, β-apo-8'-carotenal, capaxanthin, bixin, canthaxanthin, lutein, zeaxanthin or other xanthophylls. Such pigments may be present in amounts such as 0.0001-10 g, preferably 0.001-1 g, more preferably 0.01-0.1 g, per kg of the feed as a whole.

The carbohydrase incorporated into the animal feed is preferably one or more of an α-galactosidase, an amylase (such as an α-amylase or gluco-amylase) or a non-starch polysaccharidase. In case the carbohydrase is a non-starch

polysaccharidase, then this is preferably at least one of a xylanase, a β-glucanase, a cellulase, a pectinase and a mannanase.

If the non-starch polysaccharidase is a xylanase, then this can be obtained from a bacteria such as *Bacillus*, *Streptomyces*, *Clostridium*, *Thermonospora*, *Microtetraspora*, or *Ruminococcus*. However, it is more preferred that the xylanase is obtained from a fungus such as *Trichoderma*, *Aspergillus*, *Humicola* or *Neocallimastix*. It is particularly preferred that the xylanase is the low pI xylanase (pI = 5.2) and/or the high pI xylanase (pI = 9.0) obtained from *Trichoderma longibrachiatum* obtainable by the method of Example 22 of WO 92/06209. It is particularly preferred that the xylanase is the high pI xylanase. The xylanase may be a mutant xylanase having an amino acid sequence not found in nature, such a sequence corresponding to that of a naturally occurring xylanase modified by inserting, deleting or replacing one or more amino acid residues in the naturally occurring xylanase.

In the case that the non-starch polysaccharidase is either a β-glucanase or a cellulase, such an enzyme can be obtained from *Trichoderma*. If the non-starch polysaccharidase is a pectinase, then it can be obtained from *Aspergillus*. A suitable mannanase can be obtained from *Caldocellum*, *Aspergillus*, *Pseudomonas*, *Trichoderma* or *Streptomyces*.

If the carbohydrase is an amylase such as an α-amylase, then this can be obtained from a *Bacillus* species including *Bacillus subtilis* and *Bacillus amyloliquefaciens*.

If the carbohydrase is an α-galactosidase, then this can be obtained from either *Aspergillus* or *Trichoderma*.

According to an alternative aspect of the invention, the enzyme incorporated in the non-viscous animal feed is a protease. Suitable protease enzymes may be obtained from *Rhizopus, Penicillium, Bacillus, Pseudomonas* or most preferably *Aspergillus*.

It is preferred that the protease is a subtilisin which can be derived from the genus *Bacillus*, such as the strains including but not limited to *Bacillus lentus, Bacillus alcalophilus, Bacillus licheniformis, Bacillus subtilis or Bacillus amyloliquefaciens*.

Suitable proteases include but are not limited to the following commercially available proteases: NOVO NEUTRASE (TM) (commercially available from Novo Nordisk); BIOFEED PRO (commercially available from Novo Nordisk); PURAFECT (TM) (commercially available from Genencor International, Inc); SAVINASE (TM) (commercially available from Novo Nordisk); MAXACAL (TM) (commercially available from Gist-Brocades); DURAZYM (TM) (commercially available from Novo Nordisk); and MAXAPEM (TM) (commercially available from Gist-Brocades).

The subtilisin may also be a mutant subtilisin having an amino acid sequence not found in nature, such a sequence corresponding to that of a naturally occurring subtilisin modified by inserting, deleting or replacing one or more amino acid residues in the naturally occurring subtilisin. Suitable mutant subtilisins are described in EP-A-0 130 756 (corresponding to US-Re-34 606) (including mutations at position +155, +104, +222, +166, +33, +169, +189, +217, +156, +152); EP-A-0 251 446; WO 91/06637 etc. The most preferred subtilisin is a mutant subtilisin which comprises a substitution at the amino acid residue position equivalent to

tyr+217 of the subtilisin obtainable from *Bacillus amyloliquefaciens* with leucine.

Methods of producing such mutant subtilisins are described in detail in the publications US-Re-34 606 and EP-A-0 251 446.

The animal feed for use in the present invention may include a small content of a feed substrate normally considered to be viscous such as wheat, rye, triticale, barley and/or oats. However, the total content of such viscous substrates should be less in weight terms than the content of the non-viscous substrates.

It is preferred that the animal feed provided for use in the present invention should comprise less than 20% by weight of water, preferably 5-15% by weight of water.

The carbohydrase and/or protease is included in the animal feed preferably in an amount of 1×10^{-6} - 1% by weight, more preferably 1×10^{-5} - 0.5% by weight, even more preferably 1×10^{-5} - 0.1% by weight, and most preferably 1×10^{-5} - 0.01% by weight of enzyme protein based on the animal feed as a whole.

The animal feed whose use is provided by the present invention can be prepared in a number of different ways. For instance, a solution of the carbohydrase and/or the protease is applied to an extruded, expanded or pelleted animal feed having appropriate non-viscous feed and water contents. The solution of the enzyme may be formed by dissolving it in a suitable solvent such as water to an appropriate concentration of, for example, 5 mg of enzyme protein per ml of solvent. This aqueous solution can then be sprayed on the pellets so that

the resulting pellets are dosed with the desired amount of enzyme such as 1 x 10^{-6} - 1% by weight.

An alternative method of preparing the animal feed is firstly to apply the carbohydrase and/or protease to a physiologically acceptable carrier as described in EP-A-0 257 996. This enzyme bearing carrier is then mixed with the other components constituting the animal feed. The resulting mixture is then extruded, expanded or pelleted into animal feeds having a content of non-viscous components of at least 15% by weight, a water content of less 20% by weight and 1 x 10^{-6} - 1% by weight of the enzyme. The physiologically acceptable carrier in the above embodiment is preferably one or more of milled wheat, barley, maize, oats, rye, soya or a by-product of any thereof. The enzyme bearing carrier is added in an amount of e.g. 0.01-50 g per kilo of the other components of the animal feed.

According to the use provided by the present invention, the inclusion of the enzyme enables increased pigment uptake from a feed by healthy poultry, fish or crustaceans. In the context of the present description, the term "poultry" includes, but is not limited to, chickens, turkeys, geese and ducks. The use provided by the present invention can enhance pigmentation of broiler skin, the pigmentation of egg yolks, the pigmentation of fish flesh and skin, or the pigmentation of crustacean flesh. Each of these enhancements of pigmentation results in a product having improved market value. In particular, it has been found that consumers in general appreciate chicken whose skin has a pleasing yellow pigmentation, and appreciate salmonids such as salmon and trout whose flesh has a pleasing pink colouration.

According to a further aspect of the present invention, a non-viscous animal feed is provided which comprises:

a) at least 15% by weight of one or more of:

(i) a cereal selected from sorghum, rice, millet, maize and a by-product of any thereof,

(ii) oilseeds selected from soya, canola, rapeseed, pea, bean, peanut, sunflower, safflower, lupin, flax and a by-product of any thereof, and

(iii) a root crop selected from tapioca, cassava and a by-product of either thereof;

(b) less than 20% by weight of water;

(c) 1×10^{-6} - 1% by weight of a protease and optionally a carbohydrase; and

(d) 1×10^{-5} - 1% by weight of one or more pigments.

In a final aspect, the present invention provides a method for increasing the absorption by fish, crustaceans or healthy poultry of a pigment present in their feed comprising:

(a) at least 15% by weight of one or more of:

(i) a cereal selected from sorghum, rice, millet, maize and a by-product of any thereof,

(ii) oilseeds selected from soya, canola, rapeseed, pea, bean, peanut, sunflower, safflower, lupin, flax and a by-product of any thereof, and

(iii) a root crop selected from tapioca, cassava and a by-product of either thereof; and

(b) less than 20% by weight of water;

the method comprising the step of incorporating in the feed 1×10^{-6} - 1% by weight of a carbohydrase and/or a protease.

In the above aspects relating to an animal feed and a method for increasing the bio-availability of a pigment present in an animal feed, all of the preferred aspects previously described in connection with the use-aspect of the present invention remain applicable.

The present invention will now be further explained by way of the following Examples.

Example 1

Four different groups each comprising 320 healthy Arbor Acre male broiler chickens were respectively fed *ad libitum* from 0 to 49 days of age with pellets of the four sorghum-based feeds set out in the following Table 1

Table 1

Ingredients	Feed 1	Feed 2	Feed 3	Feed 4
Sorghum	647.7	646.7	553.5	552.5
Soybean Meal	168	168	36	36
Fullfat Soybean	75	75	75	75
Canola	-	-	200	200
Fishmeal	50	50	50	50
Dicalcium phosphate	11	11	12	12
Soybean Oil	9	9	7	7
Xanthophyll	4.1	4.1	4.09	4.09
Salt	2	2	2	2
Vitamin mix	2	2	2	2
Methionine	1.6	1.6	0.7	0.7
Mineral mix	1	1	1	1
Sodium Bicarbonate	1.3	1.3	1.3	1.3
Choline	0.9	0.9	0.9	0.9
Maxiban	0.5	0.5	0.5	0.5
3 Nitro	0.45	0.45	0.45	0.45
Lysine	0.3	0.3	1.4	1.4
Endox	0.1	0.1	0.1	0.1
Flavomycine	0.05	0.05	0.05	0.05
Red Carophyl	0.05	0.05	0.02	0.02
Enzyme Premix	-	1	-	1

Relative amounts of the various components in the above Table are in terms of g/kg of the feed as a whole. The enzyme pre-mix comprises a wheat-based carrier containing sufficient enzymes such that Feeds 2 and 4 each include respectively around 0.0005, 0.001 and 0.001 g per kg of feed of a xylanase, an α-amylase and a protease. The xylanase is the high pI xylanase obtainable from *Trichoderma longibrachiatum;* the protease is obtainable from *Bacillus subtilis;* and the α-amylase is obtainable from *Bacillus amyloliquefaciensis*.

A computer analysis of the nutritional values of Feeds 1-4 is set out in the following Table 2.

Table 2

Feed	Feed 1	Feed 2	Feed 3	Feed 4
Crude Protein %	20	20	19.9	19.9
Lysine %	1.1	1.1	1.1	1.1
Methionine %	0.5	0.5	0.45	0.45
Met. & Cys.	0.82	0.82	0.82	0.82
Calcium	0.98	0.98	1	1
Phosphorus	0.41	0.41	0.41	0.41
ME kcal/kg	3151	3151	3148	3148

After the 49th day, the broilers were sacrificed and their level of skin pigment measured. This measurement was carried out by firstly scalding the broiler carcasses at 54°C. Feathers were then removed from the chest and abdomen, and the abdominal vein located. Skin pigmentation was then measured by Minolta colorimeter post-processing on a C300 model in which the measuring gun is held next to the skin at the

abdominal vein, and the reading taken. The gun measures L* (brightness), and C* (chroma). Brightness is a measure of the dispersion of the pigmented particles within the region of measurement such that the higher the value, the greater the saturation of the pigment. Chroma measures the red colour "*a*" and yellow colour "*b*". Chroma can be calculated according to the equation $C^* = \sqrt{(a^2 + b^2)}$.

The results of these measurements for the four feeds are set out in the following Table 3.

Table 3

	Brightness*	Chroma*
Feed 1	64.19	31.20
Feed 2	62.57	32.00
Feed 3	65.00	30.50
Feed 4	64.40	33.00

It can be understood from the results set out in Table 3 that skin colour is enhanced in each of the enzyme supplemented Feeds 2 and 4 compared to the respective control Feeds 1 and 3. This enhancement is particularly evident in Feed 4 which includes a relatively high content of canola (200 g/kg). In this instance, an 8% increase in skin pigmentation was measured.

Example 2

Three different groups each comprising 36 rainbow trout having a weight of approximately 135 g were respectively fed *ad libitum* for 56 days with pellets of the Feeds 5-7 set out in the following Table 4 until they reached a weight of 260-320 g.

Table 4

	Feed 5	Feed 6	Feed 7
Low Temperature Fishmeal	52.98	14.25	14.25
Soybean Meal (44%)	-	60	60
Extruded Wheat	22.71	10.02	10.02
Marine Oil	16.46	11.03	11.03
Cellulose	4	-	-
Binder	2	2	2
Carophyll Pink (TM)	0.005	0.005	0.005
Mineral mix	0.25	0.25	0.25
Vitamin mix	0.75	0.75	0.75
Choline	0.24	0.24	0.24
Vitamin C additive	0.6	0.6	0.6
DL-Met.	-	0.66	0.66
Enzyme Pre-mix	-	-	0.2

Relative amounts of the various components in the above Table are in terms of wt% of the feed as a whole. The enzyme pre-mix comprises a wheat based carrier containing sufficient enzyme such that Feed 7 includes around 0.0001 g of enzyme protein per kg of feed of each of a protease and an α-galactosidase. The protease is obtainable *Aspergillus niger* and the α-galactosidase is obtainable from *Aspergillus phoenicus*.

A computer analysis of the nutritional value of the Feeds 5-7 is set out in the following Table 5

Table 5

	Feed 5	Feed 6	Feed 7
Crude Protein	40	40	40
Crude Fat	21.98	13.15	13.15
Fibre	3.6	3.6	3.6
Gross Energy kcal/kg	4871	4871	4871

At the end of the trial, flesh colour was measured by HPLC analysis of the carotenoid pigment concentration in the flesh. Feeds 5-7 respectively resulted in 10.6, 8.7 and 10.3 mg carotenoid pigment (astaxanthin) per kg of fish flesh.

It can be understood from a comparison of Feeds 6 and 7 that carotenoid concentration per kg of fish flesh was increased by 18% in the case of the enzyme supplemented Feed 7 compared to the control Feed 6.

Example 3

Four different groups each comprising 200 healthy Arbor Acre male broiler chickens were respectively fed *ad libitum* from 0 to 53 days of age with mash formed from either the standard or low density feeds set out in the following Table 6. Two of the groups were control groups respectively fed the standard and low density feeds without enzyme supplementation. The other two groups were fed the same standard or low density feeds supplemented by an enzyme premix.

Table 6

Ingredients g/kg	Standard Starter	Low Density Starter	Standard Grower	Low Density Grower	Standard Finisher	Low Density Finisher
Maize	453	429	525	518	590	579
Soybean meal 44	266	292	215	233	154	181
Full-fat soya	177	75	200	116	200	109
Wheat Midds	50	150	-	80	-	80
Meat & bone meal	24	23	24	23	21	21
Vegetable Oil	20	20	27	20	25	20
Vit/Min/ Amino acid Premix	10	11	10	10	10	10
Enzyme Premix	0 or 1	0 or 1	0 or 1	0 or 1	0 or 1	0 or 1

Relative amounts of the various components in the above Table are in terms of g/kg of the feed as a whole. Starter feeds were fed for days 0-21, grower feeds were fed for days 22-42, and finisher feeds were fed for days 43-53. The enzyme premix which is added to the feeds of two groups of the broilers comprises a wheat-based carrier containing sufficient enzymes such that the starter, grower and finisher feeds all include respectively around 0.0005 g/kg of xylanase protein, 0.001 g/kg of α-amylase protein and 0.001 g/kg of protease protein; the xylanase, α-amylase and protease being the same as described in Example 1.

A computer analysis of the nutritional value of the above feeds is set out in the following Table 7.

Table 7

	Standard Starter	Low Density Starter	Standard Grower	Low Density Grower	Standard Finisher	Low Density Finisher
AME (kcal/kg)	3350	3180	3500	3325	3550	3375
Crude Protein %	21.5	20.4	20	19	18	17.1
Lysine %	1.1	1.02	1.03	0.95	0.9	0.83
Met + Cys%	0.76	0.71	0.76	0.72	0.61	0.58

After the 53rd day, the broilers were sacrificed and their skin pigmentation measured. This measurement was carried out in the same manner as described in Example 1 except that skin pigmentation was measured by Minolta colorimeter post-processing on a CR 200-B Chroma Meter. The results of these measurements for the feeds set out in Table 6 are set out in the following Table 8.

Table 8

	Brightness*	Chroma*
Standard Diet	67.74	15.10
Standard Diet + Enzyme	68.39	16.11
Low Density Diet	68.94	15.19
Low Density Diet + Enzyme	67.98	16.94

It can be understood from the results set out in Table 8 that pigmentation of the broiler skin is enhanced by incorporating the enzyme premix containing xylanase, protease and α-amylase in maize-soybean diets. When the enzyme premix was added to the standard diet, then skin colour was increased by 7%. When the enzymes were added to the low nutrient density diet, then this improved skin colour by 7.5%.

The results set out in the above Examples 1-3 demonstrate how the use of a carbohydrase and/or a protease as a component of a non-viscous animal feed helps to promote the absorption of pigments from the feed by an animal. Accordingly, these results demonstrate the advantages achievable according to the present invention.

The effects demonstrated above of enhancing pigment uptake in accordance with the object of the present invention can also

be obtained with feeds based upon other non-viscous feed substrates such as rice, millet, rapeseed, pea, bean, peanut, sunflower, safflower, lupin, millet, flax, tapioca or cassava. Such effects of enhanced skin pigmentation can also be obtained in crustaceans as well as in the exemplified healthy poultry and fish.

CLAIMS:

1. The use of an enzyme selected from a carbohydrase and/or a protease as a component of a non-viscous animal feed for promoting the absorption of pigments present in the feed by fish, crustaceans or healthy poultry.

2. The use according to Claim 1, wherein the non-viscous animal feed comprises at least 15% by weight of one or more of:

(i) a cereal selected from sorghum, rice, millet, maize and a by-product of any thereof,

(ii) oilseeds selected from soya, canola, rapeseed, pea, bean, peanut, sunflower, safflower, lupin, flax and a by-product of any thereof, and

(iii) a root crop selected from tapioca, cassava and a by-product of either thereof.

3. The use according to Claim 1 or Claim 2, wherein the pigment is one or more carotenoid pigments.

4. The use according to Claim 3, wherein the carotenoid pigment is one or more of astaxanthin, lycopene, β-carotene, citraxanthin, capsorubin, β-apo-8'-carotenoic acid ethyl ester, β-apo-8'-carotenal, capaxanthin, bixin, canthaxanthin, lutein, zeaxanthin and a xanthophyll.

5. The use according to any preceding Claim, wherein the carbohydrase is an α-galactosidase, an amylase, or a non-starch polysaccharidase.

6. The use according to Claim 5, wherein the non-starch polysaccharidase is at least one of a xylanase, a β-glucanase, a cellulase, a pectinase and a mannanase.

7. The use according to Claim 6, wherein the xylanase is the low pI xylanase and/or the high pI xylanase obtainable from *Trichoderma longibrachiatum*.

8. The use according to any preceding Claim, wherein the protease is obtainable from *Aspergillus*, or the protease is a subtilisin or mutant thereof obtainable from the genus *Bacillus*.

9. The use according to any preceding Claim, wherein the feed comprises 1 x 10^{-6}-1% by weight of the enzyme.

10. A method for increasing the absorption by fish, crustaceans or healthy poultry of a pigment present in their feed comprising:

(a) at least 15% by weight of one or more of:
  (i) a cereal selected from sorghum, rice, millet, maize and a by-product of any thereof,
  (ii) oilseeds selected from soya, canola, rapeseed, pea, bean, peanut, sunflower, safflower, lupin, flax and a by-product of any thereof, and
  (iii) a root crop selected from tapioca, cassava and a by-product of either thereof; and
(b) less than 20% by weight of water;

the method comprising the step of incorporating in the feed 1 x 10^{-6} - 1% by weight of a carbohydrase and/or a protease.

11. A non-viscous animal feed comprising:

(a) at least 15% by weight of one or more of:

(i) a cereal selected from sorghum, rice, millet, maize and a by-product of any thereof,

(ii) oilseeds selected from soya, canola, rapeseed, pea, bean, peanut, sunflower, safflower, lupin, flax and a by-product of any thereof, and

(iii) a root crop selected from tapioca, cassava and a by-product of either thereof;

(b) less than 20% by weight of water;

(c) 1×10^{-6} - 1% by weight of a protease and optionally a carbohydrase; and

(d) 1×10^{-5} - 1% by weight of one or more pigments.

12. A non-viscous animal feed according to Claim 11, wherein the carbohydrase is an α-galactosidase, an amylase, or a non-starch polysaccharidase.

13. A non-viscous animal feed according to Claim 12, wherein the non-starch polysaccharidase is at least one of a xylanase, a β-glucanase, a cellulase, a pectinase and a mannanase.

14. A non-viscous animal feed according to Claim 13, wherein the xylanase is the low pI xylanase and/or the high pI xylanase obtainable from *Trichoderma longibrachiatum*.

15. A non-viscous animal feed according to any of Claims 11-14, wherein the protease is obtainable from *Aspergillus*, or the protease is a subtilisin or mutant thereof obtainable from the genus *Bacillus*.

INTERNATIONAL SEARCH REPORT

Internat. Application No
PCT/EP 97/05964

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23K1/16 A23K1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 05739 A (FINNFEEDS INT LTD ;GENENCOR INT (US)) 29 February 1996 cited in the application see page 20, paragraph 2 - page 28, paragraph 3 see example 5 see claims 1-20,22-27	11-15
Y	EP 0 682 874 A (EWOS AB) 22 November 1995 see page 2, line 50 - line 53 see claims 1,14,15	11-15
A	WO 91 04673 A (NOVONORDISK AS ;GUYOMARC H NUTRITION ANIMALE (FR)) 18 April 1991 cited in the application see page 1, line 7 - line 21 see page 2, line 6 - line 13 see page 8, line 1 - page 11, line 18	1-7,9,10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
5 March 1998	18/03/1998

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INTERNATIONAL SEARCH REPORT

Internati Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	DATABASE WPI Section Ch, Week 9143 Derwent Publications Ltd., London, GB; Class C03, AN 91-316444 XP002057812 & SU 1 629 008 A (ODESS FOOD IND TECH) , 23 February 1991 see abstract ---	11
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